

Article

Soil Microbial Functions Linked Fragrant Rice 2-Acetyl-1-Pyrroline with Soil Active Carbon Pool: Evidence from Soil Metagenomic Sequencing of Tillage Practices

Xiangwen Huang^{1,2,3,†}, Jiajun Lin^{1,†}, Qihuan Xie¹, Jingdan Shi¹, Xiaoxu Du¹, Shenggang Pan^{1,2,3}, Xiangru Tang^{1,2,3}  and Jianying Qi^{1,2,3,*} 

- ¹ State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources, College of Agriculture, South China Agricultural University, Guangzhou 510642, China; hxw-2016@stu.scau.edu.cn (X.H.); sum2yang@outlook.com (J.L.); xqh456119@stu.scau.edu.cn (Q.X.); jingdan07@stu.scau.edu.cn (J.S.); xiaoxu0401@stu.scau.edu.cn (X.D.); panshenggang@scau.edu.cn (S.P.); tangxr@scau.edu.cn (X.T.)
- ² Scientific Observing and Experimental Station of Crop Cultivation in South China, Ministry of Agriculture and Rural Affairs, Guangzhou 510642, China
- ³ Guangzhou Key Laboratory for Science and Technology of Fragrant Rice, Guangzhou 510642, China
- * Correspondence: qi@scau.edu.cn
- † These authors contributed equally to this work.

Abstract: Improved tillage management in fragrant rice cropping systems can enhance soil organic carbon (SOC) and the content of 2-Acetyl-1-Pyrroline (2-AP), a crucial volatile compound contributing to the aroma of fragrant rice. Despite this, the interplay between 2-AP content in fragrant rice and SOC metabolism, alongside the influences exerted by soil microbial functions, remains poorly understood. This study introduces a comprehensive 6-year field experiment which aims to correlate SOC with rice grain 2-AP content by analyzing soil microbial KEGG functions, such as carbon and amino acid metabolism, using metagenomic sequencing. The experiment assessed three tillage practices, conventional tillage (CT), reduced tillage (RT), and no tillage (NT), with soil samples collected on three dates in 2022. The results indicated that NT significantly ($p < 0.05$) enhanced SOC content and modified carbon metabolism by upregulating the Calvin cycle (K01601) and reducing hemicellulose degradation (K01710). Additionally, NT notably increased the soil levels of alkaline amino acids, such as histidine and ornithine, which were 165.17% and 1218.42% higher, respectively, than those in CT, possibly linked to an increase in soil pH. Furthermore, the 2-AP content in fragrant rice under NT was significantly higher by 52.02% and 13.90% compared to under RT and CT, respectively. NT also upregulated K00250 (alanine, aspartate, and glutamate metabolism) and K00290 (valine, leucine, and isoleucine biosynthesis), leading to significantly higher levels of 2-AP biosynthesis-related amino acids proline and glutamate in fragrant rice grain. This study links SOC and 2-AP biosynthesis via soil microbial functions, presenting a novel strategy for improving the quality of fragrant rice through soil management practices.

Keywords: metagenomics; tillage practice; soil organic carbon; soil amino acids; microbial function; 2-Acetyl-1-Pyrroline



Citation: Huang, X.; Lin, J.; Xie, Q.; Shi, J.; Du, X.; Pan, S.; Tang, X.; Qi, J. Soil Microbial Functions Linked Fragrant Rice 2-Acetyl-1-Pyrroline with Soil Active Carbon Pool: Evidence from Soil Metagenomic Sequencing of Tillage Practices. *Agronomy* **2024**, *14*, 1308. <https://doi.org/10.3390/agronomy14061308>

Academic Editor: Yong-Xin Liu

Received: 30 March 2024

Revised: 14 June 2024

Accepted: 14 June 2024

Published: 17 June 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Soil, a cornerstone of Earth's ecosystems, shapes agricultural outcomes and the global ecological landscape. Its carbon (C) component is critical in the global cycles of C and nitrogen (N) [1], bolstering ecosystem stability, advancing sustainable agricultural methods, and influencing climate change dynamics [2–4]. Soil microorganisms, key facilitators of soil organic carbon (SOC) breakdown and conversion, play a pivotal role in the cycling and renewal of organic matter [5]. Their activity crucially contributes to the C flux within

ecosystems, impacting the feedback loop between climate and the global C cycle while also playing a significant role in nutrient cycling, ecosystem functionality, and soil vitality [6–9].

Metagenomics, employing high-throughput sequencing to analyze microbial genomes and functions, has become an invaluable approach for examining soil C and N cycling, biodiversity, and ecosystem services [10–13]. This technique has shed light on the positive effects of no tillage (NT) on microbial biomass, community diversity, and functionality [14–16]. Studies revealed a dominance of fungi in soils under various management practices, with significant differences in microbial community compositions, such as increased abundances of specific bacterial groups in NT systems incorporating crop residues [17,18].

Rice, making up a substantial portion of China's grain output, is vital for global food security [19]. Elevated consumer standards for rice quality, in terms of flavor and aroma, have heightened demand for aromatic rice varieties. These varieties, celebrated for their distinctive scent, superior taste, and nutritional value, are favored by consumers and farmers alike [20–22]. The 2-AP is the main component contributing to the aromatic characteristics of aromatic rice [23–26], which is influenced by various factors such as agricultural management [9,23,27] and climatic conditions [28]. Particularly, soil carbon metabolism and amino acid metabolism play critical roles in the generation of 2-AP. Amino acids are precursors of the 2-AP; thus, foliar application of amino acids is considered as important management in increasing 2-AP [23].

Despite the acknowledged importance of amino acids in the synthesis of 2-AP and their absorption by rice plants, the relationship between soil amino acid metabolism and 2-AP production in aromatic rice systems has yet to be fully explored. As a dynamic component of SOC, soil amino acids are speculated to be a key factor in increasing 2-AP levels in rice. Its availability is influenced by soil organic carbon (SOC) and microbial activity. SOC supports microbial activity by providing organic matter, which, in turn, promotes the release and availability of amino acids. Microorganisms play a crucial role in the decomposition of organic matter and nutrient cycling, and their activity and diversity directly impact the metabolic processes of soil amino acids [29,30]. This potential highlights the need for in-depth studies of their role within the fragrant rice cropping system aiming not only to deepen our understanding of soil carbon metabolism but also to refine aromatic rice production practices to align with consumer preferences.

Noticeably, based on multiple years of data, our previous study surprisingly showed that NT could increase fragrant rice yield and aroma (2-AP) [31,32]. However, the underlying driving factors regulating 2-AP under NT is not clear. We also found NT in a double rice cropping system increased SOC and soil total nitrogen (TN) [32], which might contribute to soil amino acid metabolism and assimilation by fragrant rice. Soil carbon metabolism is central to soil health and fertility, providing energy and nutrients for soil microorganisms through the decomposition and transformation of organic matter [33,34]. The level of SOC directly affects microbial activity and diversity, thereby influencing the production and availability of amino acids in the soil [35,36]. NT practices reduce soil disturbance, promote organic matter accumulation and microbial diversity, and thus improve soil structure and increase SOC content [37,38]. In this environment, microbial communities become more active, enabling more efficient decomposition of organic matter and the release of more amino acids [39,40]. Additionally, proline and ornithine are important precursor amino acids for 2-AP synthesis. NT practices improve the soil environment, significantly increasing the levels of these amino acids [41,42]. Therefore, we hypothesize that NT could increase fragrant rice 2-AP by upregulating soil C metabolism and soil amino acid metabolisms. In these metabolic processes, the generated amino acids can be absorbed by rice root. Soil C metabolism could generate CO₂ and contribute to greenhouse gas emissions in the atmosphere, but improved C metabolism is able to increase soil nutrition release (e.g., amino acid) and change the soil amino acid content and metabolisms. These pathways could be linked and annotated by using the KEGG database. Based on the connection provided by soil microbial KEGG functions, SOC and fragrant rice 2-AP are closely related; thus, this study suggests a new mechanism for improving fragrant rice aroma by using soil.

2. Materials and Methods

2.1. Experimental Site and Design

The field experiment commenced in 2017 at the Field Experiment Station of the College of Agriculture, South China Agricultural University, located at coordinates 23°13' N, 113°81' E, with an elevation of 11 m. This area is characterized by a subtropical monsoon climate featuring an annual average maximum temperature of 27 °C, a minimum of 19 °C, as recorded in 2022. The experiment was conducted on sandy loam soil. Initial physicochemical analysis of the soil in 2017 revealed a pH of 6.56. The SOC and TN concentrations were measured to be 8.75 g·kg⁻¹ and 1.1 g·kg⁻¹, respectively. Additionally, the soil's available nitrogen, phosphorus, and potassium content were assessed, with values of 53.72 mg·kg⁻¹, 16.37 mg·kg⁻¹, and 120.08 mg·kg⁻¹, respectively, providing a foundational understanding of the site's fertility and condition for the experimental work.

This experiment site is a designated field experiment site, and, from 2017 to 2022, the experiments were conducted following the planting and management patterns described in this study. In 2022, the investigation into soil characteristics covered a depth of 0–20 cm, obtaining the data shown in Table 1. The study was delineated into three distinct tillage treatments to evaluate their effects on soil and crop dynamics, conventional tillage (CT), reduced tillage (RT), and no tillage (NT), with each plot spanning 100 m² (5 by 20 m). A uniform deep side fertilization strategy was employed across all treatments, applying a base fertilizer at a concentration of 600 kg per hectare. This blend comprised 15% nitrogen (N), 4% phosphorus pentoxide (P₂O₅), 6% potassium oxide (K₂O), and 10% organic matter, resulting in application rates of 90 kg N ha⁻¹, 24 kg P₂O₅ ha⁻¹, and 36 kg K₂O ha⁻¹, incorporated into the soil at a depth of 10 cm.

Table 1. Soil basic properties of the field experiment.

Treatments	Clay (%)	pH	TP (g kg ⁻¹)	TK (g kg ⁻¹)	AK (mg kg ⁻¹)	AP (mg kg ⁻¹)	NH ₄ -N (mg kg ⁻¹)	NO ₃ -N (mg kg ⁻¹)
NT	19.44	5.59 (0.06)	0.77 (0.01)	22.48 (0.07)	20 (2)	48.58 (0.19)	4.30 (0.13)	0.82 (0.05)
RT	11.27	5.20 (0.03)	0.68 (0.01)	22.99 (0.35)	17 (1)	30.42 (1.34)	1.75 (0.05)	0.69 (0.03)
CT	15.15	5.04 (0.03)	0.69 (0.01)	25.76 (0.44)	20 (1)	36.96 (0.71)	2.38 (0.24)	0.53 (0.04)

Note: values in the bracket indicate the standard deviation (*n* = 3).

CT was characterized by two rounds of rotary tillage, whereas RT was limited to a single round, with both practices reaching a tillage depth of approximately 10–15 cm. In contrast, NT maintained rice stubble on the surface without any soil disturbance. The aromatic rice varieties selected for cultivation were Qingxiangyou 19 and Meixiangzhan 2. Prior to planting, an herbicide named “Nongda”, which contains 41% glyphosate, was applied. Weed removal occurred 15 days before transplanting, at a time when the paddy field was submerged under 3–4 cm of water. To ensure uniformity, a rice field laser leveler was employed once to level the field and compress the stubble. All subsequent field management practices were consistently applied across the different treatment plots.

2.2. Soil Samples Collection and Physiochemical Properties Analysis

Soil samples were gathered in April, July, and October 2022, preceding the early rice transplanting and subsequent to the harvests of both early and late rice crops. For each treatment, soil samples from 0–10 cm depth were randomly collected from three points using a shovel, placed in sealed plastic bags, and transported with ice packs to the laboratory. In total, nine samples were collected (three treatments, three replicates) with 1 kg from each plot. The soil samples were divided into two portions; visible straw and stones were removed from one portion, which was then stored in a −80 °C freezer for microbial measurements, while the other portion was naturally air-dried, sieved to 2 mm, and then subjected to physicochemical property analysis. The physicochemical properties of the soil are shown in Table 1.

The air-dried soil samples were sieved through a 0.25 mm mesh for the analysis of soil TN and SOC. These samples were pretreated with a 4 M hydrochloric acid solution to eliminate the interference of inorganic C. An elemental analyzer (Vario Macro Cube, Elementar, Frankfurt am Main, Germany.) was used to analyze the soil TN and SOC. The soil pH was measured using a Mettler Toledo SevenEasy benchtop pH meter (Greifensee, Switzerland) at a solid-to-liquid ratio of 1:2.5. Soil dissolved organic carbon (DOC) was determined by employing unsieved, field-moist soil samples. The extraction process involved shaking for 1 h with a 0.5 M K₂SO₄ solution at a temperature of approximately 20 °C using a soil-to-solution ratio of 1:5 (*w/v*) [43].

2.3. Soil DNA Extraction, 16S, Internal Transcribed Spacer, and Metagenomic Sequencing

In April and July, soil samples were analyzed to ascertain bacterial and fungal diversity and composition with a focus on the abundance of the bacterial 16S ribosomal RNA (rRNA) and fungal internal transcribed spacer (ITS) rRNA genes. This analysis was conducted in triplicate using a quantitative real-time PCR (qPCR) detection system (ABI prism 7900, Applied Biosystem, Waltham, MA, USA). For bacterial studies, the 16S rRNA genes' V3–V4 regions were amplified using specific primers: the forward primer ACTCCTACGGGAG-GCAGCA and the reverse primer GGACTACHVGGGTWTCTAAT. These primers were chosen due to their ability to target highly conserved regions flanking the variable regions, ensuring comprehensive coverage of the bacterial community. For fungal analyses, the ITS V1 region was amplified using the forward primer GGAAGTAAAAGTCGTAACAAGG and the reverse primer GCTGCGTTCTTCATCGATGC, selected for their specificity and effectiveness in capturing a wide range of fungal taxa. Following PCR amplification, product quantification was achieved with the Quant-iT PicoGreen dsDNA Assay Kit and measured using a Microplate reader (FLx800, BioTek, Winooski, VT, USA). This method ensured accurate and reliable quantification of DNA, essential for subsequent analyses.

The resultant amplicons were pooled in equal proportions and subjected to Single-Molecule Real-Time (SMRT) sequencing on the PacBio Sequel platform, facilitated by Shanghai Personal Biotechnology Co., Ltd., Shanghai, China. The generated sequencing data have been deposited in the NCBI database under the accession numbers PRJNA928576 and PRJNA928566, ensuring accessibility for further analysis and review by the scientific community.

The process of extracting DNA from environmental samples was meticulously performed to ensure the integrity and quality of the genomic DNA for subsequent analyses. Following the completion of genomic DNA extraction, the purity and concentration of the extracted DNA were assessed through 1% agarose gel electrophoresis, a crucial step in verifying the success of the extraction process and the suitability of the DNA for further processing. To facilitate next-generation sequencing, the genomic DNA was fragmented into approximately 400 base pair (bp) pieces using the Covaris M220 system, a method chosen for its precision and reproducibility in DNA shearing. The NEXTFLEX™ Rapid DNA-Seq Kit was then employed to construct sequencing libraries from the fragmented DNA, a critical phase in preparing the samples for high-throughput sequencing. Subsequent sequencing of the metagenome utilized second-generation sequencing technology and was conducted on the Illumina platform—a choice reflecting the technology's high accuracy, efficiency, and capacity for generating vast quantities of sequence data. This sequencing employed either NovaSeq Reagent Kits or HiSeq X Reagent Kits depending on the specific requirements of the sequencing run. The generated raw sequence data have been duly submitted to the NCBI database, where they are accessible under the accession number PRJNA995040. This deposit ensures that the data are available for review, analysis, and reuse by the scientific community, facilitating further research and discovery in the field of environmental genomics.

To enhance the quality of the raw sequencing data, Fastp was employed for trimming, yielding high-quality reads for further analysis. This tool is accessible at <https://github.com/OpenGene/fastp> (accessed on 27 December 2023). Following data clean-

ing, the assembly of the metagenome was performed using Megahit, as described by [44] (<https://github.com/voutcn/megahit>, accessed on 27 December 2023) and Newbler (<https://ngs.csr.uky.edu/Newbler>, accessed on 27 December 2023). Only genes with a nucleotide length of 100 bp or more were selected for further analysis. These selected genes were then translated into amino acid sequences to compile a statistical table of gene prediction results for each sample. The ORFs in the contigs of the splicing results were predicted using Prodigal [45]. CD-HIT software [46] (<http://www.bioinformatics.org/cd-hit/>, accessed on 27 December 2023) was used for clustering (default parameters: 90% identity, 90% coverage). To create a non-redundant gene set, the longest gene from each category was chosen as the representative sequence. SOAPaligner software [47] (<http://soap.genomics.org.cn/>, accessed on 27 December 2023) was used to compare each sample's high-quality reads with the non-redundant gene set (default parameter: 95% identity), and the abundance information of the gene in the corresponding sample was calculated. DIAMOND [48] (<https://github.com/bbuchfink/diamond>, accessed on 27 December 2023) was used to annotate the non-redundant gene set with the NR database (parameters: blastp; E-value $\leq 1 \times 10^{-5}$) and KEGG's gene database (GENES) (parameters: blastp; E-value $\leq 1 \times 10^{-5}$) for functional annotation. Mothur (v.1.30.1, http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity, accessed on 27 December 2023) index analysis was used, and the OTU similarity level for index evaluation was 97% (0.97).

2.4. Detection of 2-Acetyl-1-Pyrroline and Yield of Aromatic Rice

During the mature period of aromatic rice, 10 rice plants were randomly selected, and 30 leaves were taken and placed in an incubator with an ice box. Grain samples (10 g) were ground into powder, and then the content of 2-AP was measured by simultaneous distillation extraction (SDE) and analyzed by GCMS-QP 2010 Plus (Shimadzu, Kyoto, Japan). The detection method was referred to in [49]. Each treatment was fixed and cut to 1 square meter, which was repeated 3 times, dried after threshing, and dried after air selection to remove impurities and empty grains. Then, the actual yield was weighed and converted into mu yield, and the unit used is kg.

2.5. Data Analysis

The R software package (version: 4.2.1) was used to analyze SOC, TN, and pH to investigate the impact of different cultivation management practices on soil C dynamics ($p < 0.05$). A PCoA analysis was also carried out to explore the effects of different cultivation management practices on soil microbial diversity. The Shannon index was utilized to measure α -diversity, and significant differences between the two groups were identified using the statistical t -test (FDR, Kruskal–Wallis rank-sum test). Except for Circos diagrams (Circos-0.67-7 (<http://circos.ca/>, accessed on 27 December 2023)) and co-occurrence network analysis (using the Networkx package in Python 3.9.0 64-bit v2024.4.1), all graphs were created with SigmaPlot 12.5 and the R software package 4.3.0.

3. Results

3.1. Soil Basic Properties, Microbial Diversity, and Composition

Compared to the other tillage practices, NT significantly increased SOC, TN, and pH ($p < 0.05$). NT showed a gradual decreasing trend in SOC and TN over time and an increasing pH (Figure 1A–C). During the sampling season in April and July, there was no significant impact of tillage practices on bacterial and fungal α -diversity (Figure 1D,E).

NT significantly improved the α -diversity of the soil microbiome, as indicated by metagenomic sequencing, compared to other treatments during the sampling season in October (Figure 1F). The Shannon index, a measure of α -diversity, was notably higher under NT conditions. Although the bacterial and fungal compositions, assessed by Principal Coordinates Analysis (PCoA), did not show significant changes due to tillage practices (Figure 1G,H), the overall structure of the soil microbial community was distinctly different under NT compared to CT and RT (Figure 1I). This indicates that, while specific microbial

taxa did not vary greatly, the broader microbial community structure was significantly influenced by NT, likely due to the changes in soil environment and resource availability induced by NT practices.

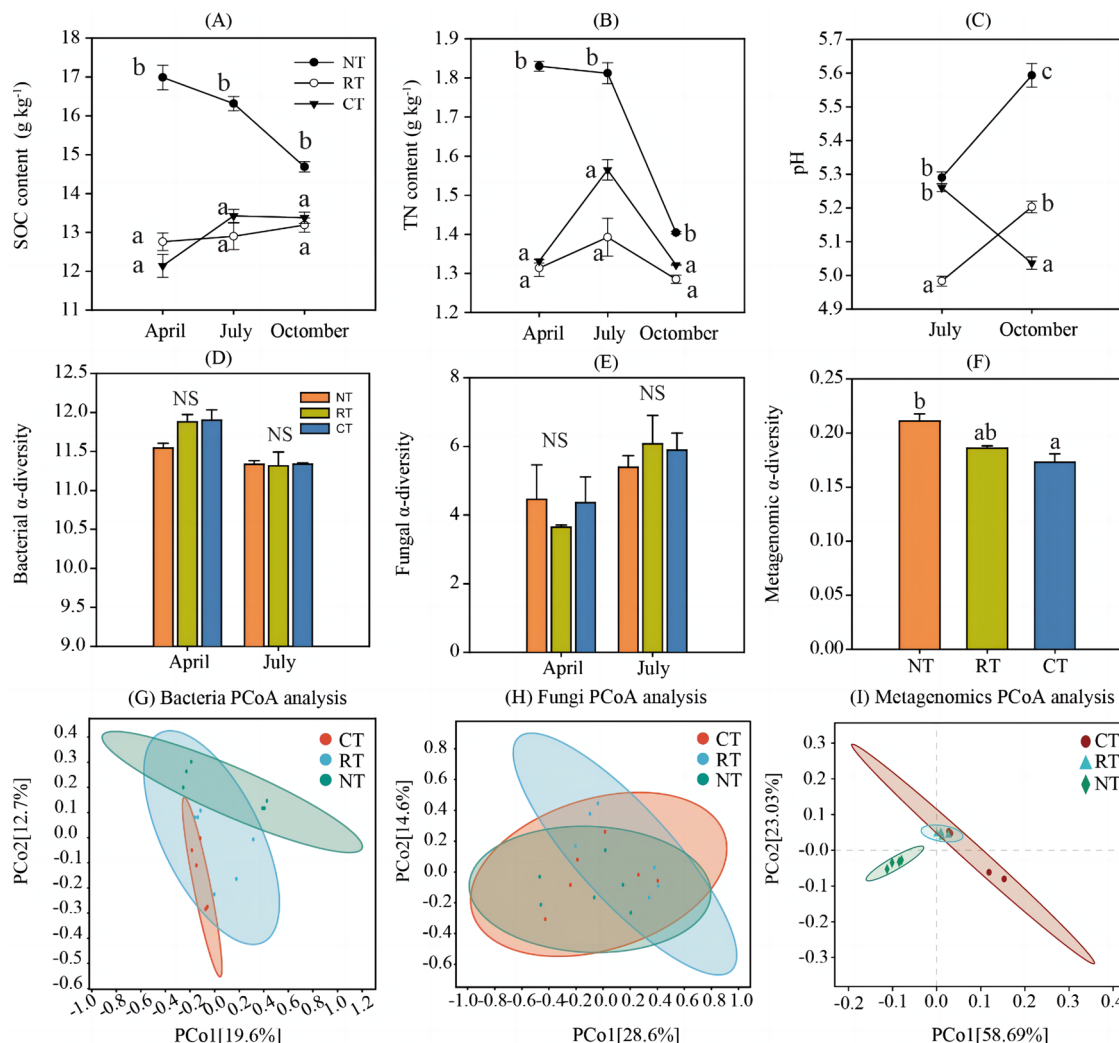


Figure 1. Soil carbon dynamics and microbial diversity affected by contrasting tillage management methods. (A–C) represent the dynamics of soil organic carbon (SOC), soil total nitrogen (TN), and soil pH during three sampling seasons. (D–F) represent the α-diversity of bacteria, fungi, and metagenome (expressed by Shannon index). (G–I) represent the Principal Coordinates Analysis (PCoA) based on bacteria, fungi (combined data of April and July), and metagenome (data from October). NT, no tillage; RT, reduced tillage; CT, conventional tillage. Error bars in (A–F) represent the standard errors ($n = 3$ for (A–E), and $n = 4$ for (F)). “NS” indicates no significant difference. For each parameter, different letters indicate significant differences between means at $p < 0.05$.

At the species level, there were 27,333, 27,776, and 27,678 microbial species under NT, RT, and CT treatments, respectively. Each treatment had unique species (1402, 1143, and 1546 species, respectively), and 23,162 species were shared among the three treatments (Figure 2A). Under NT, RT, and CT treatments, there were 164, 163, and 162 KEGG pathways at level 3 of Metabolism, respectively, with 162 species shared among all three treatments and 1, 0, and 0 unique species for each treatment, respectively. Additionally, Euryarchaeota, Candidatus_Thermoplasmatot, and Planctomycetes were predominantly observed under NT, while Cyanobacteria and Chloroflexi were more abundant under the CT. RT and NT significantly impacted soil microbial diversity compared to CT. The NT had the highest abundance of Euryarchaeota, Candidatus_Thermoplasmatot, and Planctomycetes, and the

lowest abundance of Cyanobacteria and Chloroflexi (Figure 2C). The order of homology for the major subunits of 3-oxoacyl-[acyl-carrier protein] reductase, arginine synthase, and formate dehydrogenase was CT < RT < NT. Compared to CT, RT and NT increased the relative abundance of genes related to adenylate cyclase (Figure 2D).

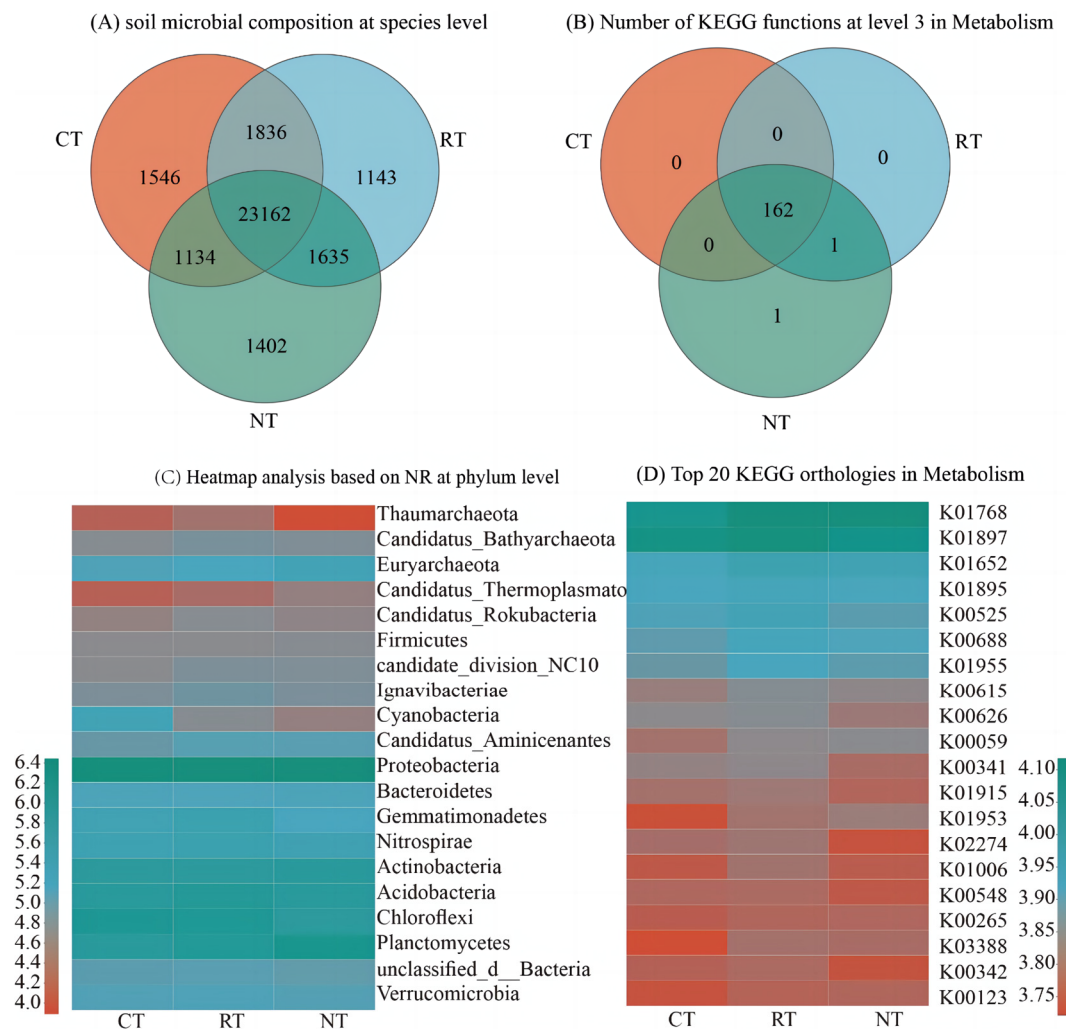


Figure 2. Effects of tillage practices on soil microbial composition and KEGG orthology. (A,B) are Venn plots of microbial species and KEGG function in Metabolism. (C) represents the heatmap analysis of soil microbial composition at phylum level. (D) represents the heatmap analysis of KEGG orthology in Metabolism. NT, no tillage; RT, reduced tillage; CT, conventional tillage.

3.2. Soil Carbon and Methane Metabolism

The enzyme 2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase subunit alpha [EC: 1.2.7.3 1.2.7.11] (K00174) had the highest abundance, followed by isoamylase [EC:3.2.1.68] (K01214) and 2-oxoacid ferredoxin oxidoreductase subunit beta [EC:1.2.7.3 1.2.7.11] (K00175). Different tillage practices had significant impacts on three soil C metabolism-related enzymes: 2-oxoacid ferredoxin oxidoreductase subunit beta [EC:1.2.7.3 1.2.7.11] (K00175), dTDP-glucose 4,6-dehydratase [EC:4.2.1.46] (K01710), and ribulose-bisphosphate carboxylase large chain [EC:4.1.1.39] (K01601) (Figure 3A). The gene sequence of trimethylamine-corinoid protein Co-methyltransferase [EC:2.1.1.250] (K14083) in the soil methane metabolism significantly differed under different tillage treatments (Figure 3B). Compared to traditional methods, the NT treatment had the highest abundance of Planctomycetes, and there were significant differences in the top 15 microbial species' abundance under different tillage treatments (Figure 3C). There were also significant differences in the top

15 KEGG functional abundances of microbial communities at different taxonomic levels under different tillage treatments, with microbial metabolism being the highest proportion among different environments (Figure 3D).

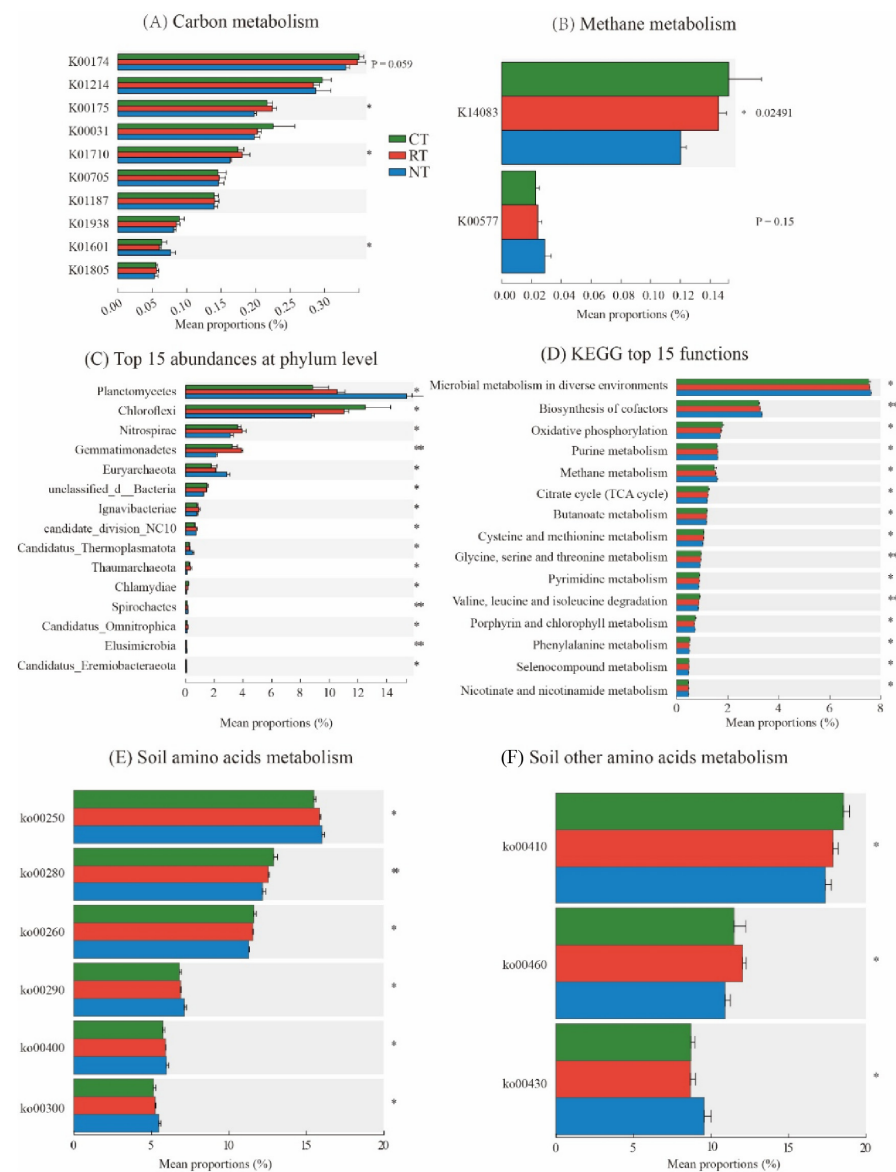


Figure 3. Effects of tillage practices on soil carbon and methane metabolisms. (A) represents the functions of carbon metabolisms. (B) represents the methane metabolisms. (C,D) represent the top 15 abundances at phylum level based on NR and KEGG functions (at pathway level 3). (E,F) represents soil amino acid metabolism and other amino acid metabolism. Error bars represent the standard errors ($n = 4$). $0.01 < p \leq 0.05$ *, $0.001 < p \leq 0.01$ **. NT, no tillage; RT, reduced tillage; CT, conventional tillage. The KEGG names and KO descriptions are shown in the Supplementary Materials.

For the soil amino acid metabolism, NT significantly increased Ko00250 (Alanine, aspartate, and glutamate metabolism), Ko00290 (Valine, leucine, and isoleucine biosynthesis), Ko00400 (Phenylalanine, tyrosine, and tryptophan biosynthesis), and Ko00300 (Lysine biosynthesis) compared with RT or CT (Figure 3E). Additionally, the other amino acids' metabolism can be affected by NT, e.g., that of Ko00410 (beta-Alanine metabolism), Ko00460 (Cyanoamino acid metabolism), and Ko00430 (Taurine and hypotaurine metabolism) (Figure 3F).

3.3. Soil Alkaline Amino Acids Content and Total Carbon Content of Amino Acids

Under NT conditions, the contents of alkaline amino acids lysine and arginine in fragrant rice were $2023.67 \mu\text{mol kg}^{-1}$ and $817.89 \mu\text{mol kg}^{-1}$, respectively, showing significant increases of 63.1% to 84.7% and 26.2% to 82.5% compared to under CT (Figure 4A,E). The content of histidine in rice grain did not change significantly with tillage practices (Figure 4C). Additionally, the content of lysine and arginine in the soil under NT did not differ significantly from that under RT and CT (Figure 4B,F). The histidine content in soil under NT was $4.79 \mu\text{mol kg}^{-1}$, which was 165.1% to 183.3% higher than in other tillage practices ($p < 0.05$), while the ornithine content under NT was $7.5 \mu\text{mol kg}^{-1}$, higher than $0.4 \mu\text{mol kg}^{-1}$ under RT and $0.6 \mu\text{mol kg}^{-1}$ under CT (Figure 4D,G). Tillage practices did not significantly affect the soil C content in total amino acids, with amino-acid-derived C accounting for 27.9% to 49.6% of dissolved organic carbon (DOC) (Figure 4H,I).

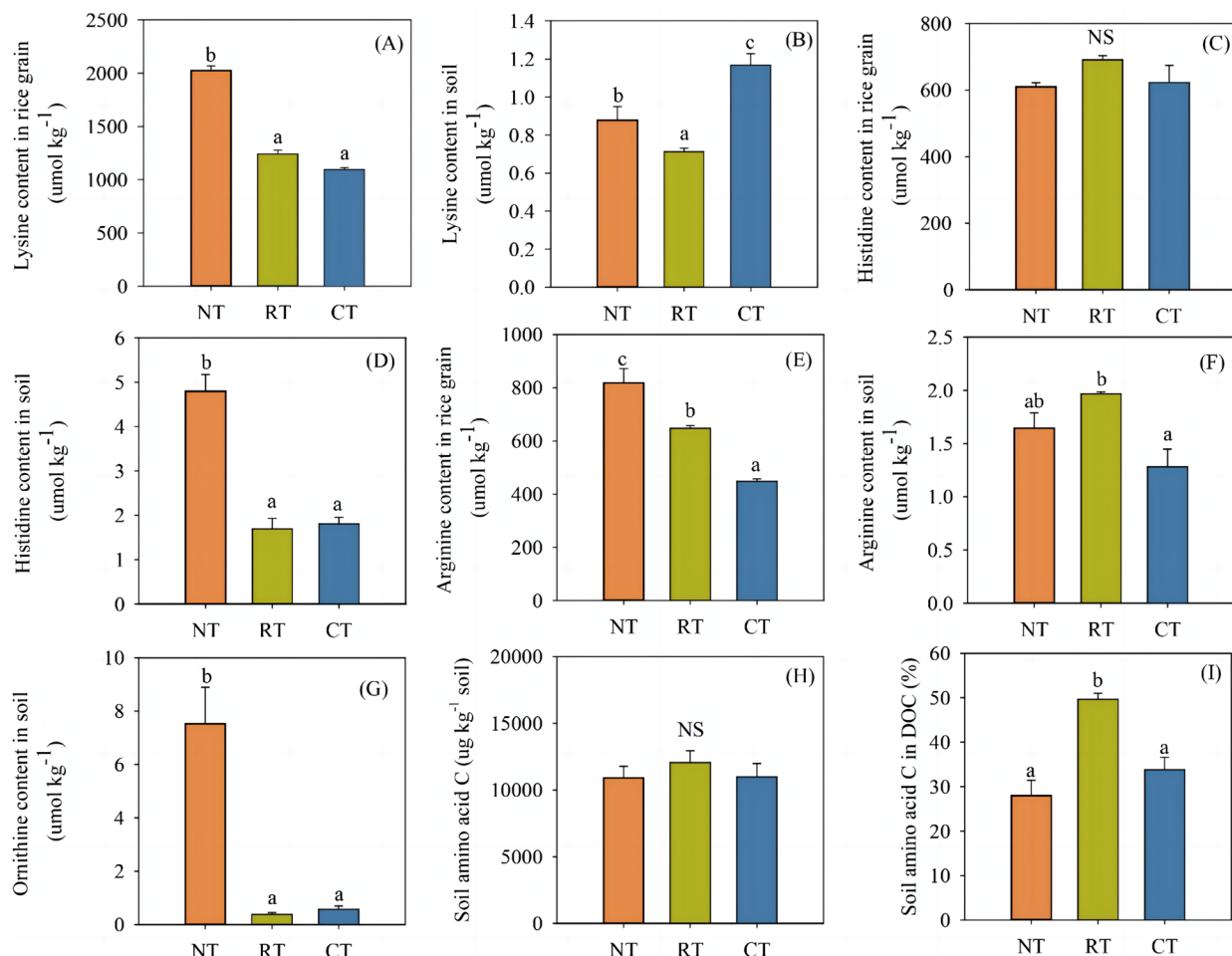


Figure 4. Effects of contrasting tillage management methods on soil alkaline amino acids content and total carbon content of amino acids. (A) represents the content of lysine in rice. (B) represents the content of lysine in soil. (C) represents the content of Histidine in rice. (D) represents the content of lysine in soil. (E) represents the content of Arginine in rice. (F) represents the content of Arginine in soil. (G) represents the content of Ornithine in soil. (H) represents soil amino acid C. (I) represents soil amino acid C in DOC. NT, no tillage; RT, reduced tillage; CT, conventional tillage. Error bars represent the standard errors ($n = 3$). “NS” indicates no significant difference. For each parameter, different letters indicate significant differences between means at $p < 0.05$.

3.4. Fragrant Rice Yield, 2-Acetyl-1-Pyrroline, and Related Amino Acids Content

NT significantly ($p < 0.05$) increased fragrant rice yield by 17.4% and 21.3% compared with RT and CT (Figure 5A). Under NT, the content of 2-AP in fragrant rice was

295.64 $\mu\text{g kg}^{-1}$, significantly higher than under RT and CT, with increases of 52.02% and 13.90%, respectively (Figure 5B). The contents of amino acids related to 2-AP biosynthesis, such as proline and glutamate, were 461.9 and 416.5 $\mu\text{g kg}^{-1}$ in rice under NT, significantly higher than under RT and CT. Specifically, proline content was 32.94% and 72.51% higher, and glutamate content was 22.38% and 26.21% higher compared to under RT and CT, respectively (Figure 5C,E). There were no significant differences in soil proline and glutamate under NT compared to the other two tillage practices (Figure 5D,F).

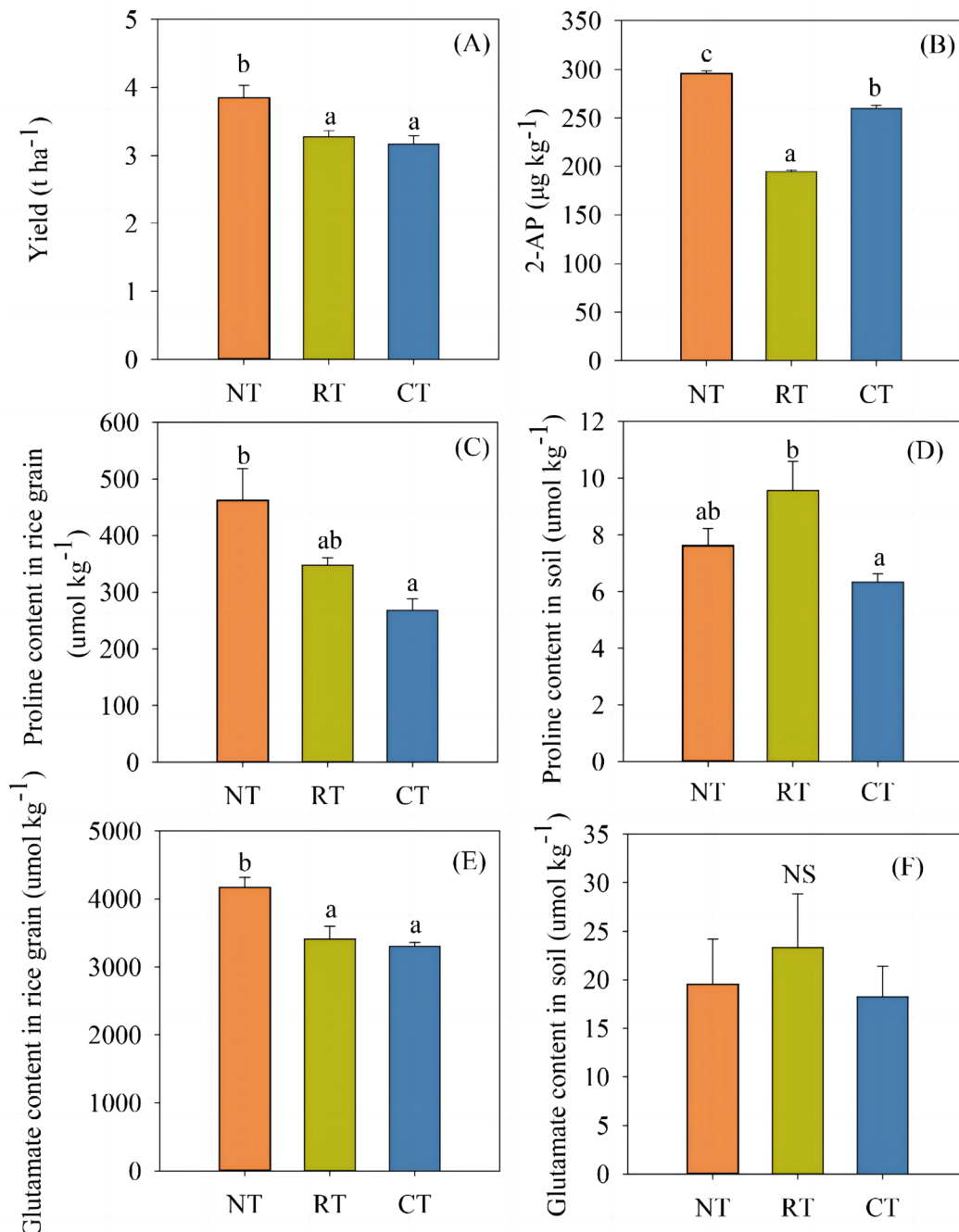


Figure 5. Effects of contrasting tillage management methods on fragrant rice yield, 2-Acetyl-1-Pyrroline (2-AP), and 2-AP-related amino acids. NT, no tillage; RT, reduced tillage; CT, conventional tillage. Error bars represent the standard errors ($n = 3$). “NS” indicates no significant difference. For each parameter, different letters indicate significant differences between means at $p < 0.05$.

In addition, fragrant rice grain 2-AP content was significantly positively correlated with SOC, TN, yield, and Global and overview maps (Figure 6A–D), while it was significantly negatively correlated with secondary metabolism (Figure 6E). The SOC was significantly negatively correlated with amino acid metabolism and other amino acid metabolism but significantly positively correlated with Global and overview maps (Figure 6F). Rice yield was significantly positively correlated with SOC (Figure 6I).

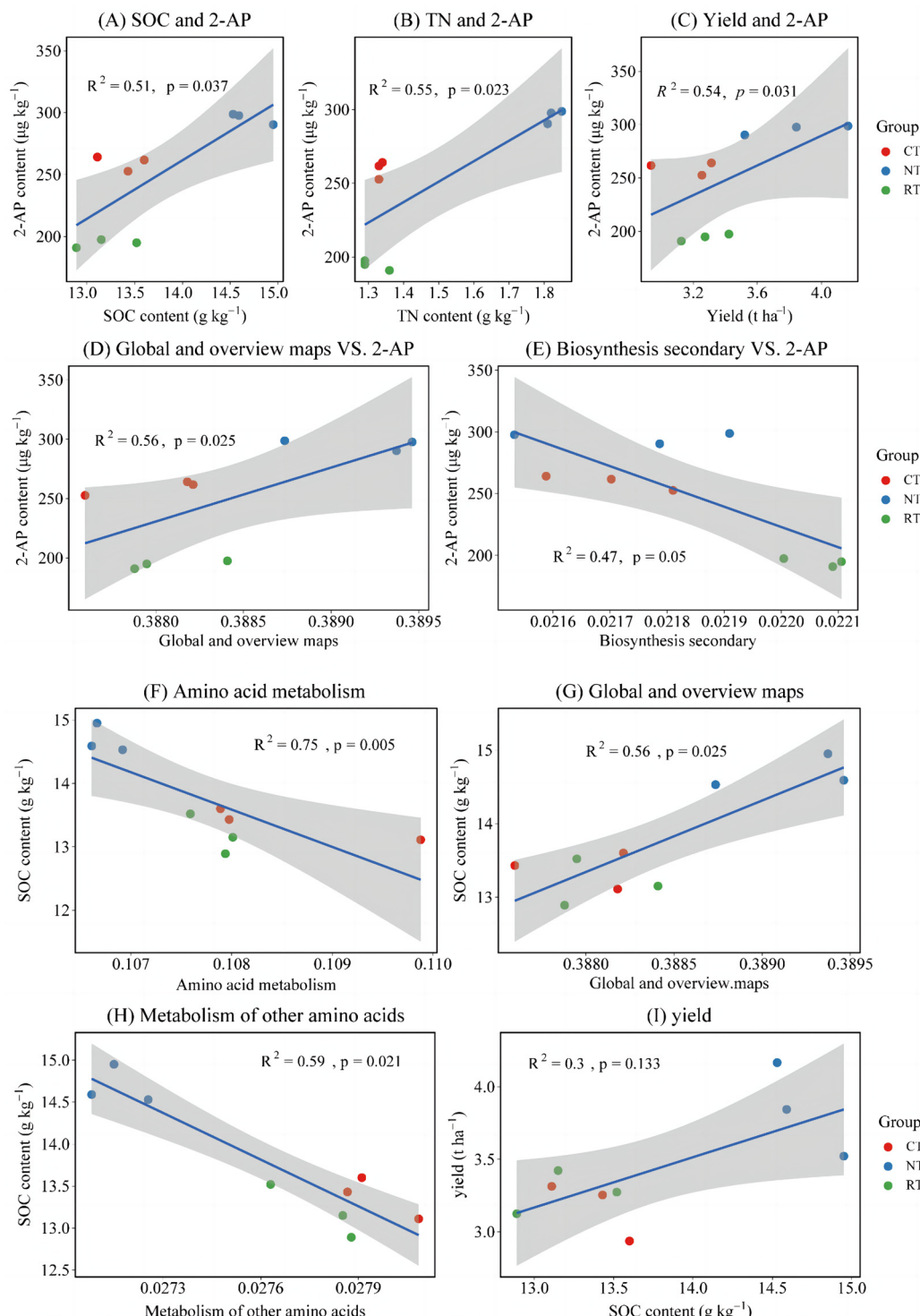


Figure 6. The relation of soil microbial functions to fragrant rice 2-Acetyl-1-Pyrroline (2-AP) content and soil organic carbon (SOC).

4. Discussions

4.1. Effects of Tillage Practices on Soil Chemical and Microbial Properties

Higher SOC and TN contents were recorded in the NT compared with the CT and RT treatments, which is consistent with the findings of Qi et al. [32]. The reduced soil disturbance and improved rice-residue-derived C input might contribute to the increase in SOC and TN under NT. Under NT conditions, soil pH increases over time due to the formation of a stable soil structure driven by the accumulation of organic matter and microbial activity. This stable structure enhances soil aggregation, water infiltration, and aeration, reducing the accumulation of acidic substances. Additionally, the retention of basic cations such as calcium, magnesium, and potassium further contributes to the rise in soil pH. Regarding the soil microbe, it was found that the metagenomic α -diversity of soil microorganisms under NT was significantly higher than that under conventional tillage, which is consistent with the results of [50]. Furthermore, we observed an increased proportion of Planctomycetes under NT compared with the other treatments. Planctomycetes are widely distributed in various ecosystems and are believed to play an important role in soil ecosystems [51,52]. Previous studies have shown that some Planctomycetes species are capable of catalyzing ammonification, which promotes nitrogen cycling in the soil [53,54].

4.2. Effects of Tillage Practices on KEGG Functions in Soil

Different tillage practices exerted significant influences on numerous metabolic pathways within soil [55–57]. We found that NT notably impacted carbon (C) metabolism by reducing the presence of 2-oxoacid ferredoxin oxidoreductase subunit beta (EC:1.2.7.3) and dTDP-glucose 4,6-dehydratase (EC:4.2.1.46) while increasing the abundance of ribulose-bisphosphate carboxylase large chain (EC:4.1.1.39). Furthermore, NT had a significant effect on methane metabolism by decreasing the levels of trimethylamine corrinoid protein Co-methyltransferase (EC:2.1.1.250) in comparison to conventional tillage [58]. Simultaneously, when compared to CT, NT significantly elevated the concentrations of the alkaline amino acids (e.g., lysine and arginine) in rice grains, while histidine content remained notably consistent [59]. Additionally, the content of lysine and arginine in the soil did not exhibit significant differences between CT and NT; however, the histidine content under NT surpassed that in other tillage practices. In the context of soil amino acid metabolism, NT significantly augmented Ko00250 (alanine, aspartate, and glutamate metabolism), Ko00290 (valine, leucine, and isoleucine biosynthesis), Ko00400 (phenylalanine, tyrosine, and tryptophan biosynthesis), and Ko00300 (lysine biosynthesis) compared to CT, affecting various other amino acid metabolic pathways such as Ko00410 (beta-alanine metabolism), Ko00460 (cyanoamino acid metabolism), and Ko00430 (taurine and hypotaurine metabolism). A previous study has also demonstrated that NT influences methane emissions through soil microorganisms [60]. Nevertheless, methane emissions were not assessed in paddy fields during our study; thus, further research is essential to elucidate the mechanisms by which NT regulates methane emissions and the correlation between methane emissions and soil microorganism activity.

4.3. Effects of Tillage Practices on Yield and 2-Acetyl-1-Pyrroline of Fragrant Rice

In the present study, higher levels of 2-AP and yield were observed under NT compared to the other tillage practices. This finding is consistent with the data from years of continuous observation, as previously shown by Du et al. [31]. NT significantly increased the content of 2-AP in aromatic rice by regulating SOC and amino acid metabolism, indicating that SOC and amino acid metabolism play an important role in 2-AP synthesis. The reason for the yield increase under NT was mainly derived from the combined rice cultivation technology, e.g., the pot seeding transplanter used to protect the rice root during transplanting, synchronized fertilization at the 0–10 cm soil depth, and the land laser leveler used for rice stubble pressing before rice transplanting under NT, as summarized by Qi et al. [32]. The increased 2-AP content could be attributed to the regulation in amino acid metabolism. Amino acids including proline, ornithine, and glutamate are important

precursors in 2-AP biosynthesis in fragrant rice, as reported by a previous study [61]. In addition, we observed a positive and significant relationship between 2-AP content and soil TN content (Table 2). This finding is consistent with previous studies, which showed that application of nitrogen fertilizer significantly increased the 2-AP content of fragrant rice grain [62,63]. Moreover, we observed a significant and positive relationship between 2-AP content and SOC content. NT significantly increased SOC and TN contents, and changed the diversity and structure of soil microorganisms. In particular, the increase in the content of alkaline amino acids (such as histidine and ornithine) in soil under NT may be due to the increase in soil pH. However, the effects of SOC on 2-AP biosynthesis in fragrant rice have not been extensively reported, and further studies should be carried out to explore the relationship between 2-AP and SOC.

Table 2. Correlation analysis of soil microbial functional abundances with fragrant rice yield, 2-Acetyl-1-pyrroline (2-AP) content, and soil organic carbon (SOC).

	Biosynthesis Secondary	Global and Overview Maps	Metabolism of Cofactors and Vitamins	Metabolism of Other Amino Acids	Amino Acid Metabolism
2-AP	−0.683 *	0.750 *	0.650	−0.467	−0.517
Yield	−0.217	0.767 *	0.867 **	−0.750 *	−0.483
SOC	−0.383	0.750 *	0.583	−0.767 *	−0.867 **

Notes: The soil functions were annotated by KEGG dataset (Kyoto Encyclopedia of Genes and Genomes, <http://www.genome.jp/kegg/>, accessed on 27 December 2023) at pathway level 2 in Metabolism. ** and * represent the significant level of Spearman correlation analysis at $p < 0.01$ and $p < 0.05$.

5. Conclusions

Our 6-year field experiment has demonstrated that NT management significantly enhances both the 2-AP content and yield of fragrant rice. This finding is a notable departure from traditional beliefs, which hold that tillage practices do not significantly impact rice aroma and quality. Our hypothesis that the increased 2-AP content under NT is linked to improved soil quality, particularly higher SOC, soil microbial composition, and amino acid metabolism, is supported by our results. Compared to CT and RT, NT significantly increased SOC and TN contents, positively influencing the diversity and structure of soil microorganisms. Importantly, soil alkaline amino acids (e.g., histidine and ornithine) were improved by NT, possibly due to the increased soil pH. Furthermore, many metabolic pathways engaged in carbon metabolism and amino acid metabolism were altered by tillage management. NT upregulated pathways such as K00250 (alanine, aspartate, and glutamate metabolism) and K00290 (valine, leucine, and isoleucine biosynthesis), but decreased K00280 (tryptophan metabolism) or K00260 (cysteine and methionine metabolism). These changes contributed to higher levels of 2-AP biosynthesis-related amino acids, such as proline and glutamate, in fragrant rice grains. Our analysis revealed significant positive correlations between 2-AP content and SOC and TN contents. Additionally, NT increased the abundance of key genes involved in amino acid metabolism, further supporting the role of soil amino acids and their metabolism as critical factors driving the increase in 2-AP content in fragrant rice. We therefore infer that the soil active carbon pool (i.e., soil amino acids) and its metabolism can be potential driving factors that increase fragrant rice grain 2-AP under contrasting tillage practices. In this context, SOC and fragrant rice grain 2-AP were connected by the soil amino acids metabolism of soil microbes.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14061308/s1>, Table S1: Metabolism pathways of carbon degradation annotated by KEGG; Table S2: Metabolism pathways of carbon fixation annotated by KEGG; Table S3: Metabolism pathways of methane metabolism annotated by KEGG; Table S4: Metabolism pathways of amino acids metabolism annotated by KEGG; Table S5: Metabolism pathways of other amino acids metabolism annotated by KEGG.

Author Contributions: Methodology, J.L.; Formal analysis, X.H., J.S. and X.D.; Resources, Q.X.; Writing—original draft, X.H. and J.Q.; Writing—review & editing, X.H.; Supervision, S.P. and J.Q.; Project administration, X.T.; Funding acquisition, J.Q. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Natural Science Foundation of China (grant no. 32201921) and Guangdong Basic and Applied Basic Research Foundation (2023A1515010738).

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: We thank Wei Wu (University of Edinburgh) for polishing the English language.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

Abbreviations	Full name	Abbreviations	Full name
SOC	Soil organic carbon	C	Carbon
TN	Total nitrogen	N	Nitrogen
NT	No tillage	RT	Reduced tillage
CT	Conventional tillage		

References

1. Niu, S.; Song, L.; Wang, J.; Luo, Y.; Yu, G. Dynamic Carbon-Nitrogen Coupling under Global Change. *Sci. China-Life Sci.* **2023**, *66*, 771–782. [[CrossRef](#)] [[PubMed](#)]
2. Dawson, J.J.C.; Smith, P. Carbon Losses from Soil and Its Consequences for Land-Use Management. *Sci. Total Environ.* **2007**, *382*, 165–190. [[CrossRef](#)] [[PubMed](#)]
3. de Tombeur, F.; Sohy, V.; Chenu, C.; Colinet, G.; Cornelis, J.-T. Effects of Permaculture Practices on Soil Physicochemical Properties and Organic Matter Distribution in Aggregates: A Case Study of the Bec-Hellouin Farm (France). *Front. Environ. Sci.* **2018**, *6*, 116. [[CrossRef](#)]
4. Jackson, R.B.; Lajtha, K.; Crow, S.E.; Hugelius, G.; Kramer, M.G.; Pineiro, G. The Ecology of Soil Carbon: Pools, Vulnerabilities, and Biotic and Abiotic Controls. In *Annual Review of Ecology, Evolution, and Systematics*; Futuyma, D.J., Ed.; Annual Reviews: Palo Alto, CA, USA, 2017; Volume 48, pp. 419–445. ISBN 978-0-8243-1448-4.
5. Schimel, J.P.; Schaeffer, S.M. Microbial Control over Carbon Cycling in Soil. *Front. Microbiol.* **2012**, *3*, 348. [[CrossRef](#)] [[PubMed](#)]
6. Liang, C.; Schimel, J.P.; Jastrow, J.D. The Importance of Anabolism in Microbial Control over Soil Carbon Storage. *Nat. Microbiol.* **2017**, *2*, 17105. [[CrossRef](#)] [[PubMed](#)]
7. Qiu, S.; Yang, H.; Zhang, S.; Huang, S.; Zhao, S.; Xu, X.; He, P.; Zhou, W.; Zhao, Y.; Yan, N.; et al. Carbon Storage in an Arable Soil Combining Field Measurements, Aggregate Turnover Modeling and Climate Scenarios. *Catena* **2023**, *220*, 106708. [[CrossRef](#)]
8. Semenov, A.M.; Dukic, D.A. The Role of Microbial Communities in Soil Formation and Soil Ecosystem Health. *Paleontol. J.* **2020**, *54*, 843–852. [[CrossRef](#)]
9. Wang, Y.; Wu, F.; Li, X.; Li, C.; Zhao, Y.; Gao, Y.; Liu, J. Effects of Plants and Soil Microorganisms on Organic Carbon and the Relationship between Carbon and Nitrogen in Constructed Wetlands. *Environ. Sci. Pollut. Res.* **2023**, *30*, 62249–62261. [[CrossRef](#)] [[PubMed](#)]
10. Barnett, S.E.; Egan, R.; Foster, B.; Elie-Fadrosch, E.A.; Buckley, D.H. Genomic Features Predict Bacterial Life History Strategies in Soil, as Identified by Metagenomic Stable Isotope Probing. *MBIO* **2023**, *14*, e03584-22. [[CrossRef](#)]
11. Hernandez-Guzman, M.; Perez-Hernandez, V.; Gomez-Acata, S.; Jimenez-Bueno, N.; Verhulst, N.; Catalina Munoz-Arenas, L.; Navarro-Noya, Y.E.; Luna-Guido, M.L.; Dendooven, L. Application of Young Maize Plant Residues Alters the Microbiome Composition and Its Functioning in a Soil under Conservation Agriculture: A Metagenomics Study. *Arch. Microbiol.* **2022**, *204*, 458. [[CrossRef](#)]
12. Zhong, C.; Chen, C.; Wang, L.; Ning, K. Integrating Pan-Genome with Metagenome for Microbial Community Profiling. *Comput. Struct. Biotechnol. J.* **2021**, *19*, 1458–1466. [[CrossRef](#)] [[PubMed](#)]
13. Kroeger, M.E.; Delmont, T.O.; Eren, A.M.; Meyer, K.M.; Guo, J.; Khan, K.; Rodrigues, J.L.M.; Bohannon, B.J.M.; Tringe, S.G.; Borges, C.D.; et al. New Biological Insights Into How Deforestation in Amazonia Affects Soil Microbial Communities Using Metagenomics and Metagenome-Assembled Genomes. *Front. Microbiol.* **2018**, *9*, 1635. [[CrossRef](#)] [[PubMed](#)]
14. Sun, L.; Li, J.; Wang, Q.; Zhang, Y.; Xu, Z.; Wang, R.; Wang, X.; Jia, G.; Zhang, X. The Effects of Eight Years of Conservation Tillage on the Soil Physicochemical Properties and Bacterial Communities in a Rain-Fed Agroecosystem of the Loess Plateau, China. *Land Degrad. Dev.* **2020**, *31*, 2475–2489. [[CrossRef](#)]

15. Morugan-Coronado, A.; Perez-Rodriguez, P.; Insolia, E.; Soto-Gomez, D.; Fernandez-Calvino, D.; Zornoza, R. The Impact of Crop Diversification, Tillage and Fertilization Type on Soil Total Microbial, Fungal and Bacterial Abundance: A Worldwide Meta-Analysis of Agricultural Sites. *Agric. Ecosyst. Environ.* **2022**, *329*, 107867. [\[CrossRef\]](#)
16. Zhong, S.; Zeng, H. Long-Term Interactions of Reduced Tillage and Different Amounts of Residue Retaining Improved Soil Environment in a Semi-Arid Tropical Climate. *Chil. J. Agric. Res.* **2020**, *80*, 197–208. [\[CrossRef\]](#)
17. Liu, C.; Li, L.; Xie, J.; Coulter, J.A.; Zhang, R.; Luo, Z.; Cai, L.; Wang, L.; Gopalakrishnan, S. Soil Bacterial Diversity and Potential Functions Are Regulated by Long-Term Conservation Tillage and Straw Mulching. *Microorganisms* **2020**, *8*, 836. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Mishra, A.; Singh, D.; Hathi, Z.; Purohit, H.J.; Philip, A.; Uthup, T.K.; Singh, L. Soil Microbiome Dynamics Associated with Conversion of Tropical Forests to Different Rubber Based Land Use Management Systems. *Appl. Soil Ecol.* **2023**, *188*, 104933. [\[CrossRef\]](#)
19. Huang, M. The Decreasing Area of Hybrid Rice Production in China: Causes and Potential Effects on Chinese Rice Self-Sufficiency. *Food Secur.* **2022**, *14*, 267–272. [\[CrossRef\]](#)
20. Diversity of Global Rice Markets and the Science Required for Consumer-Targeted Rice Breeding-All Databases. Available online: <https://webofscience.clarivate.cn/wos/alldb/full-record/WOS:000329925800022> (accessed on 1 June 2024).
21. Custodio, M.C.; Cuevas, R.P.; Ynion, J.; Laborte, A.G.; Velasco, M.L.; Demont, M. Rice Quality: How Is It Defined by Consumers, Industry, Food Scientists, and Geneticists? *Trends Food Sci. Technol.* **2019**, *92*, 122–137. [\[CrossRef\]](#)
22. Suwannaporn, P.; Linnemann, A.; Chaveesuk, R. Consumer Preference Mapping for Rice Product Concepts. *Br. Food J.* **2008**, *110*, 595–606. [\[CrossRef\]](#)
23. Imran, M.; Shafiq, S.; Ashraf, U.; Qi, J.; Mo, Z.; Tang, X. Biosynthesis of 2-Acetyl-1-Pyrroline in Fragrant Rice: Recent Insights into Agro-Management, Environmental Factors, and Functional Genomics. *J. Agric. Food Chem.* **2023**, *71*, 4201–4215. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Mo, Z.; Li, W.; Pan, S.; Fitzgerald, T.L.; Xiao, F.; Tang, Y.; Wang, Y.; Duan, M.; Tian, H.; Tang, X. Shading during the Grain Filling Period Increases 2-Acetyl-1-Pyrroline Content in Fragrant Rice. *Rice* **2015**, *8*, 9. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Starkenmann, C.; Niclass, Y.; Vuichoud, B.; Schweizer, S.; He, X. Occurrence of 2-Acetyl-1-Pyrroline and Its Nonvolatile Precursors in Celtuce (*Lactuca Sativa* L. Var. *Augustana*). *J. Agric. Food Chem.* **2019**, *67*, 11710–11717. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Wei, X.; Sun, Q.; Methven, L.; Elmore, J.S. Comparison of the Sensory Properties of Fragrant and Non-Fragrant Rice (*Oryza Sativa*), Focusing on the Role of the Popcorn-like Aroma Compound 2-Acetyl-1-Pyrroline. *Food Chem.* **2021**, *339*, 128077. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Ruan, S.; Luo, H.; Wu, F.; He, L.; Lai, R.; Tang, X. Organic Cultivation Induced Regulation in Yield Formation, Grain Quality Attributes, and Volatile Organic Compounds of Fragrant Rice. *Food Chem.* **2023**, *405*, 134845. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Jiang, S.; Zhang, L.; Hu, F.; Tang, X.; Du, B. Zinc Supplementation and Light Intensity Affect 2-Acetyl-1-Pyrroline (2AP) Formation in Fragrant Rice. *BMC Plant Biol.* **2023**, *23*, 194. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Luo, H.; Zhang, T.; Zheng, A.; He, L.; Lai, R.; Liu, J.; Xing, P.; Tang, X. Exogenous Proline Induces Regulation in 2-Acetyl-1-Pyrroline (2-AP) Biosynthesis and Quality Characters in Fragrant Rice (*Oryza sativa* L.). *Sci. Rep.* **2020**, *10*, 13971. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Luo, H.W.; Xing, P.P.; Liu, J.H.; Lai, R.F.; He, L.X.; Zhang, T.T.; Tang, X.R. Application of Ornithine-Induced Regulation in Yield Formation, Grain Quality and Aroma of Fragrant Rice. *Cereal Res. Commun.* **2020**, *48*, 485–492. [\[CrossRef\]](#)
31. Du, B.; He, L.; Lai, R.; Luo, H.; Zhang, T.; Tang, X. Fragrant Rice Performances in Response to Continuous Zero-Tillage in Machine-Transplanted Double-Cropped Rice System. *Sci. Rep.* **2020**, *10*, 8326. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Qi, J.-Y.; Yao, X.-B.; Lu, J.; He, L.-X.; Cao, J.-L.; Kan, Z.-R.; Wang, X.; Pan, S.-G.; Tang, X.-R. A 40% Paddy Surface Soil Organic Carbon Increase after 5-Year No-Tillage Is Linked with Shifts in Soil Bacterial Composition and Functions. *Sci. Total Environ.* **2023**, *859*, 160206. [\[CrossRef\]](#)
33. Li, D.; Wu, C.; Wu, J. Soil Fungal Community Has Higher Network Stability than Bacterial Community in Response to Warming and Nitrogen Addition in a Subtropical Primary Forest. *Appl. Environ. Microbiol.* **2024**, e00001-24. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Liao, Z.; Ye, S.; Wang, S. Large Macro-Aggregate Formation Improves Soil Bacterial Metabolic Activity and Diversity in a Chronosequence of Chinese Fir Plantations. *J. Soil Sci. Plant Nutr.* **2024**, *1–13*. [\[CrossRef\]](#)
35. Liu, L.; Liu, D.; Ding, X.; Chen, M.; Zhang, S. Straw Incorporation and Nitrogen Fertilization Enhance Soil Carbon Sequestration by Altering Soil Aggregate and Microbial Community Composition in Saline-Alkali Soil. *Plant Soil* **2024**, *498*, 341–356. [\[CrossRef\]](#)
36. Zhang, Y.; Sun, C.; Wang, S.; Xie, H.; Jiang, N.; Chen, Z.; Wei, K.; Bao, X.; Song, X.; Bai, Z. Stover and Biochar Can Improve Soil Microbial Necromass Carbon, and Enzymatic Transformation at the Genetic Level. *Glob. Chang. Biol. Bioenergy* **2022**, *14*, 1082–1096. [\[CrossRef\]](#)
37. Khmelevtsova, L.E.; Sazykin, I.S.; Azhogina, T.N.; Sazykina, M.A. Influence of Agricultural Practices on Bacterial Community of Cultivated Soils. *Agriculture* **2022**, *12*, 371. [\[CrossRef\]](#)
38. Mackay, J.E.; Bernhardt, L.T.; Smith, R.G.; Ernakovich, J.G. Tillage and Pesticide Seed Treatments Have Distinct Effects on Soil Microbial Diversity and Function. *Soil Biol. Biochem.* **2023**, *176*, 108860. [\[CrossRef\]](#)
39. Cui, J.; Yang, B.; Xu, X.; Ai, C.; Zhou, W. Long-Term Maize-Soybean Rotation in Northeast China: Impact on Soil Organic Matter Stability and Microbial Decomposition. *Plant Soil* **2024**, *1–18*. [\[CrossRef\]](#)

40. Ma, L.; Wang, X.; Fang, Y.; Vancov, T.; Jin, X.; Gao, Q.; Dong, W.; Du, Z. No-Tillage Farming for Two Decades Increases Plant- and Microbial-Biomolecules in the Topsoil Rather than Soil Profile in Temperate Agroecosystem. *Soil Tillage Res.* **2024**, *241*, 106108. [\[CrossRef\]](#)
41. Du, P.; Luo, H.; He, J.; Mao, T.; Du, B.; Hu, L. Different Tillage Induces Regulation in 2-Acetyl-1-Pyrroline Biosynthesis in Direct-Seeded Fragrant Rice. *BMC Plant Biol.* **2019**, *19*, 308. [\[CrossRef\]](#)
42. Renuka, N.; Barvkar, V.T.; Ansari, Z.; Zhao, C.; Wang, C.; Zhang, Y.; Nadaf, A.B. Co-Functioning of 2AP Precursor Amino Acids Enhances 2-Acetyl-1-Pyrroline under Salt Stress in Aromatic Rice (*Oryza Sativa* L.) Cultivars. *Sci. Rep.* **2022**, *12*, 3911. [\[CrossRef\]](#)
43. Jones, D.L.; Willett, V.B. Experimental Evaluation of Methods to Quantify Dissolved Organic Nitrogen (DON) and Dissolved Organic Carbon (DOC) in Soil. *Soil Biol. Biochem.* **2006**, *38*, 991–999. [\[CrossRef\]](#)
44. Li, D.; Liu, C.-M.; Luo, R.; Sadakane, K.; Lam, T.-W. MEGAHIT: An Ultra-Fast Single-Node Solution for Large and Complex Metagenomics Assembly via Succinct de Bruijn Graph. *Bioinformatics* **2015**, *31*, 1674–1676. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Hyatt, D.; Chen, G.-L.; LoCascio, P.F.; Land, M.L.; Larimer, F.W.; Hauser, L.J. Prodigal: Prokaryotic Gene Recognition and Translation Initiation Site Identification. *BMC Bioinform.* **2010**, *11*, 119. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Fu, L.; Niu, B.; Zhu, Z.; Wu, S.; Li, W. CD-HIT: Accelerated for Clustering the next-Generation Sequencing Data. *Bioinformatics* **2012**, *28*, 3150–3152. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Li, R.; Yu, C.; Li, Y.; Lam, T.-W.; Yiu, S.-M.; Kristiansen, K.; Wang, J. SOAP2: An Improved Ultrafast Tool for Short Read Alignment. *Bioinformatics* **2009**, *25*, 1966–1967. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Buchfink, B.; Reuter, K.; Drost, H.-G. Sensitive Protein Alignments at Tree-of-Life Scale Using DIAMOND. *Nat. Methods* **2021**, *18*, 366–368. [\[CrossRef\]](#)
49. Mo, Z.; Lei, S.; Ashraf, U.; Khan, I.; Li, Y.; Pan, S.; Duan, M.; Tian, H.; Tang, X. Silicon Fertilization Modulates 2-Acetyl-1-Pyrroline Content, Yield Formation and Grain Quality of Aromatic Rice. *J. Cereal Sci.* **2017**, *75*, 17–24. [\[CrossRef\]](#)
50. Cai, L.; Guo, Z.; Zhang, J.; Gai, Z.; Liu, J.; Meng, Q.; Liu, X. No Tillage and Residue Mulching Method on Bacterial Community Diversity Regulation in a Black Soil Region of Northeastern China. *PLoS ONE* **2021**, *16*, e0256970. [\[CrossRef\]](#)
51. Buckley, D.H.; Huangyutitham, V.; Nelson, T.A.; Rumberger, A.; Thies, J.E. Diversity of *Planctomycetes* in Soil in Relation to Soil History and Environmental Heterogeneity. *Appl. Environ. Microbiol.* **2006**, *72*, 4522–4531. [\[CrossRef\]](#)
52. Ivanova, A.A.; Kulichevskaya, I.S.; Merkel, A.Y.; Toshchakov, S.V.; Dedyshev, S.N. High Diversity of *Planctomycetes* in Soils of Two Lichen-Dominated Sub-Arctic Ecosystems of Northwestern Siberia. *Front. Microbiol.* **2016**, *7*, 2065. [\[CrossRef\]](#)
53. Fuerst, J.A.; Sagulenko, E. Beyond the Bacterium: *Planctomycetes* Challenge Our Concepts of Microbial Structure and Function. *Nat. Rev. Microbiol.* **2011**, *9*, 403–413. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Op den Camp, H.J.M.; Islam, T.; Stott, M.B.; Harhangi, H.R.; Hynes, A.; Schouten, S.; Jetten, M.S.M.; Birkeland, N.-K.; Pol, A.; Dunfield, P.F. Environmental, Genomic and Taxonomic Perspectives on Methanotrophic *Verrucomicrobia*. *Environ. Microbiol. Rep.* **2009**, *1*, 293–306. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Li, Z.; Zhang, Q.; Li, F.; Li, Z.; Qiao, Y.; Du, K.; Yue, Z.; Tian, C.; Leng, P.; Cheng, H.; et al. Soil CO₂ Emission Reduction with No-Tillage and Medium Nitrogen Fertilizer Applications in Semi-Humid Maize Cropland in North China Plain. *Eur. J. Agron.* **2023**, *147*, 126838. [\[CrossRef\]](#)
56. Wacker, T.S.; Jensen, L.S.; Thorup-Kristensen, K. Conservation Agriculture Affects Soil Organic Matter Distribution, Microbial Metabolic Capacity and Nitrogen Turnover under Danish Field Conditions. *Soil Tillage Res.* **2022**, *224*, 105508. [\[CrossRef\]](#)
57. Doyeni, M.O.; Suproniene, S.; Versulienė, A.; Meskauskienė, L.; Kadziene, G. Influence of the Long-Term Application of Management Practices (Tillage, Cover Crop and Glyphosate) on Greenhouse Gas Emissions and Soil Physical Properties. *Sustainability* **2024**, *16*, 2859. [\[CrossRef\]](#)
58. Ndour, P.M.S.; Bargaz, A.; Rchiad, Z.; Pawlett, M.; Clark, I.M.; Mauchline, T.H.; Harris, J.; Lyamlouli, K. Microbial Catabolic Activity: Methods, Pertinence, and Potential Interest for Improving Microbial Inoculant Efficiency. *Microb. Ecol.* **2023**, *86*, 2211–2230. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Harish, M.N.; Choudhary, A.K.; Kumar, S.; Dass, A.; Singh, V.K.; Sharma, V.K.; Varatharajan, T.; Dhillon, M.K.; Sangwan, S.; Dua, V.K.; et al. Double Zero Tillage and Foliar Phosphorus Fertilization Coupled with Microbial Inoculants Enhance Maize Productivity and Quality in a Maize-Wheat Rotation. *Sci. Rep.* **2022**, *12*, 3161. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Qin, X.; Li, Y.; Wan, Y.; Shi, S.; Li, Y.; Wang, H.; Gao, Q.; Ma, X.; Liu, S.; Liu, Y.; et al. Relationships between Methane Emissions and Soil Microorganisms in a Double-Rice Field in Southern Subtropical China. *J. Integr. Environ. Sci.* **2012**, *9*, 97–111. [\[CrossRef\]](#)
61. Yoshihashi, T.; Huong, N.T.T.; Inatomi, H. Precursors of 2-Acetyl-1-Pyrroline, a Potent Flavor Compound of an Aromatic Rice Variety. *J. Agric. Food Chem.* **2002**, *50*, 2001–2004. [\[CrossRef\]](#)
62. Ren, Y.; Ashraf, U.; He, L.X.; Mo, Z.W.; Wang, F.; Wan, X.C.; Kong, H.; Ran, X.L.; Tang, X.R. Irrigation and Nitrogen Management Practices Affect Grain Yield and 2-Acetyl-1-Pyrroline Content in Aromatic Rice. *Appl. Ecol. Environ. Res.* **2017**, *15*, 1447–1460. [\[CrossRef\]](#)
63. Mo, Z.; Ashraf, U.; Tang, Y.; Li, W.; Pan, S.; Duan, M.; Tian, H.; Tang, X. Nitrogen Application at the Booting Stage Affects 2-Acetyl-1-Pyrroline, Proline, and Total Nitrogen Contents in Aromatic Rice. *Chil. J. Agric. Res.* **2018**, *78*, 165–172. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.